1 Bone formation and development

—In memory of Richard N. Smith

Alastair J.S. Summerlee

1 Introduction

There are two critical phases in the development of bone. The first occurs in utero when bone tissue starts to form. Centers of ossification develop in the approximate positions that will determine the basic skeletal pattern of the adult. The fetus is born with many ossified precursors of adult bone already in place. The second phase of development occurs in postnatal life as the animal starts to grow. During this time bones elongate and change shape to assume the adult form. This phase will determine the external appearance of the animal and underlie the differences observed in physical form, for example, whether or not this animal will be mouse, man, or mastodon. But bone is not static, even when fully mature. There is a constant, if much slower, rate of modeling and remodeling that continues throughout life and is affected by a variety of external and internal factors. Before discussing the prenatal and postnatal development of bone it is important to establish some of the gross anatomical and histological features that characterize adult bone.

2 Basic anatomy of bone

Descriptive anatomy divides bones into two major groups: long bones and flat bones. Initially, this classification was based solely on the gross appearance of the types of bone. The long-bone category was extended to include two further types of bone that were neither flat nor long: short bones and irregular bones. Later, it was observed that bones of the skull (which comprise the majority of the flat bones of the body) and bones of the appendicular skeleton were derived from different embryonic tissues, which strengthened the emerging view that long and flat bones developed by different processes. During the 1980s, this classic view of bone development was challenged. Despite their apparently different embryological origins, bones throughout the body develop by an identical process, and this has important implications for the organization and management of reparative processes.

A long bone consists of a compact shaft (diaphysis), an intermediate area (metaphysis), and a terminal portion (epiphysis). Each of these areas has a specific gross appearance (**Fig. 1-1**) and histological appearance (**Fig. 1-2**). The diaphysis is a hollow cylinder of compact bone which contains a medullary cavity. In contrast, the epiphysis consists of spongy or cancellous bone surrounded by a thin eggshell of compact bone. Cancellous bone is characterized by a delicate interweaving of spicules of bone known as trabeculae. In young animals a growth plate lies between these two regions of bone. This plate consists of layers of cartilage cells and matrix, blood vessels, and newly formed bone. Uniting the growth plate to the diaphysis is an intermediate region, the metaphysis, comprising columns of spongy bone. The growth plate and the metaphyseal region represent the growth component of the bone and can be seen clearly in bones of young animals. In the adult, the plate is absent, and the cancellous bone of the epiphysis becomes continuous with the cancellous bone of the diaphysis with a small white line of compact bone between them. Limb bones are classic examples of long bones.

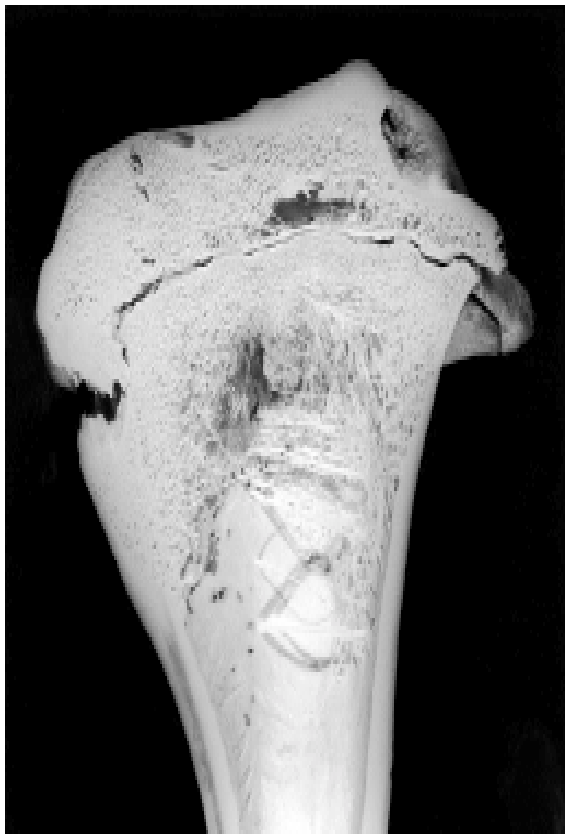

Fig. 1-1: A median section through the proximal end of an ox tibia showing the variation in the thickness of the shell of compact cortical bone and the lattice-work, honey-combed appearance of the cancellous bone. During the drying process to prepare this specimen the growth plate has separated, emphasizing the position of the epiphysis *(above)* and the metaphysis *(below).* Within the diaphysis the medullary cavity is clearly visible.

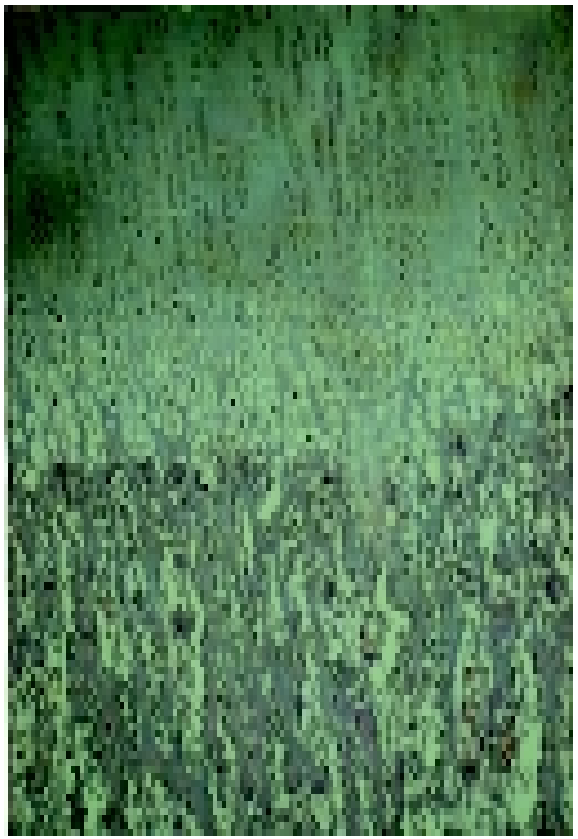

Fig. 1-2: Low-power magnification of a section through the cartilaginous growth plate between the epiphysis and the metaphysis *(below)* at the proximal end of a dog femur. A series of changes from the zones of multiplication of the cartilage *(above),* to hypertrophic layers, formation of columns, and matrix formation with partial chondrolysis to the ossifying front are shown. (H and E stain: magnification ×250; courtesy of Dr Yamashiro, Biomedical Sciences, Ontario Veterinary College.)

Flat bones are predominantly found in the skull and comprise two layers of compact bone separated by a layer of cancellous bone. Short and irregular bones consist primarily of a core of cancellous bone bounded by a cortex of compact bone of variable thickness. Many of the carpal and tarsal bones are considered to be examples of short or irregular bones.

In general, strength of bone depends on the hardness of the compact cortical bone and on the underlying scaffolding effect of the trabeculae of cancellous bone. The orientation of the trabeculae reflects the directions of maximum stresses exerted on the bone, and changes in the disposition of the mechanical forces applied to the bone will result in major remodeling of these spicules of cancellous bone.

The entire surface of bone, except where articular cartilage is present, is covered by specialized dense connective tissue known as periosteum. This layer is attached to the cortical bone below by a series of collagenous bundles known as Sharpey fibers and the strength of these attachments varies between different bones. The internal surface of bone, which includes the medullary cavity, cavities of the haversian system of compact bones and the trabeculae of cancellous bone, is lined with another connective layer, endosteum. Sandwiched between the periosteum and the outer layer of cortical bone and between the endosteum and the inner layers of bone are osteoblasts which are vital in growing bone for osteogenesis and for reparative processes throughout life. Rasmussen and Bordier [**1**] produced evidence to indicate that remodeling of bone in adult life is a very slow process, but osteoblasts below the endosteum are more active than those below the periosteum.

The histological structure of compact bone is similar for all types of bones, whether they are long, short, flat, or irregular, and reflects its mode of development. The basic construction unit is known as an osteon (haversian system). Each osteon (**Fig. 1-3**) comprises a central canal, containing blood vessels and a small amount of connective tissue, with interconnecting channels surrounded by concentric layers of bone, the lamellae. Intercalated into the bone substance are cavities with trapped osteocytes, lacunae. The lacunae communicate with each other and with the canal of the osteons through a ramifying network of canaliculae. The lacunae and canaliculae are extracellular and contain tissue fluid and interstitial substances for maintenance of the osteocytes. Presumably, therefore, nutrients and other essential molecules reach their targets by diffusion. There is a similar structural arrangement in the trabeculae of cancellous bone, but the osteons are not present.

There are three major cell types associated with mature bone: the osteoblast, which participates in the ossification process and is present when new bone is being formed; the osteoclast, which is commonly found in sites where bone is being resorbed; and the osteocyte, which is found trapped within the bone lacunae as described above and is active in constant remodeling of bone. These cell types are all derived from mesenchymal stem cells. An understanding of the lineage of osteoblasts, particularly in the postfetal skeleton, is fundamental to our appreciation of growth and reparative processes but is a subject of debate. Progenitor cells are presumably present within the marrow or in the periosteal or endosteal connective tissue, and there is some evidence to suggest that there is a continuum of cells throughout bone spaces [**2**]. Certain of these cells lie on or near the bone surface and exist as preosteoblasts, and there are indications that these are derived from specific stem cells [**3**]. The latter, however, are uncharacterized except for their potential to regenerate and differentiate into all types of progeny characteristic of the particular cell line [**4**]. There is still debate as to whether these precursors are present as part of a generalized body system of generating stromal cells or are already differentiated sufficiently to be designated specifically for the osteoblastic lineage [**5**]. Our understanding of the lineage is further complicated by the presence of fibroblastic precursor cells in the blood circulation [**6–8**]. However, fibroblastic stromal cells from certain organs, including marrow, do appear to express different antigenic markers from other organ-specific systems, which may be

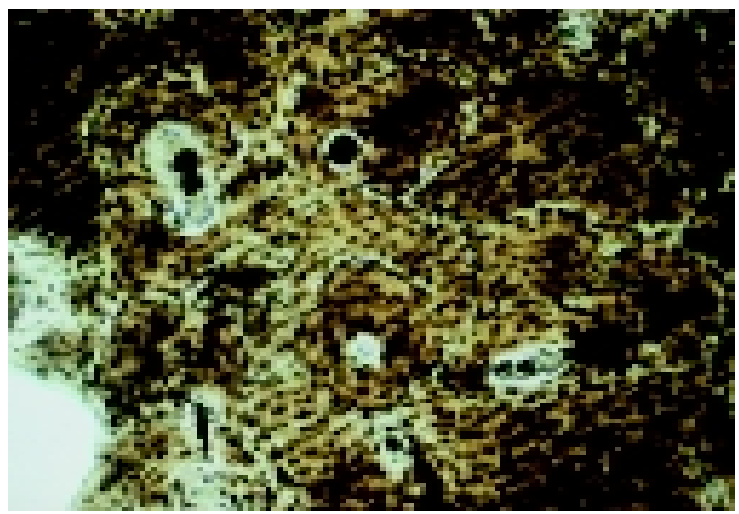

Fig. 1-3: Transverse ground section of compact bone from femoral shaft of dog. Note the variation in size and shape of osteons and their surrounding canals and the distribution of lacunae. (Courtesy of Dr Yamashiro, Biomedical Sciences, Ontario Veterinary College.)

related to functional requirements for each organ [**8**]. As will be discussed later, development of bone is dependent upon the interaction between hemopoietic and osteogenic tissues, and the possible cell lines for differentiation of the two cell populations are critical for the successful development and subsequent growth of bone. A putative lineage of stem-cell lines is shown in **Fig. 1-4**. Recent evidence compels us to reconsider the traditional view of bone formation and development and the difference between these two cell lines.

Normal bone formation occurs when "committed" stem cells and their progeny are stimulated to proliferate and differentiate. These "committed" cells are referred to as the osteogenic progenitor cells [**3**]. When they are removed mechanically with bone marrow and transplanted heterotopically they differentiate spontaneously into bone [**9–11**]. Similar cells probably reconstitute the medullary cavity following injury and ablation [**12–14**].

Fig. 1-4: Diagram to illustrate the origin and fate of cells in mature bone.
(Diagram taken from Williams P, Warwick R, Dyson M, et al., 1989, Gray's Anatomy, Churchill Livingstone.)

3 Early bone formation

Caplan et al. [**15**] described the process of development, maturation, and aging as a continuum of sequential cellular and molecular events of replacement. This is a useful concept to discuss because the changes observed in bone represent cells and matrix that are slowly and progressively replaced by structures with an ever-decreasing capacity for differentiation but with an increased degree of specialization. Some of the new structures are simply variants of their predecessor, a type of evolutionary change, while others represent the development of a novel structure that may be unique to a particular site. Whatever the process, there are three fundamental principles that govern these changes [**16**]:

1) The genomic repertoire of the organism sets the limits of the developmental and maturational possibilities. The shape, size, and presence of particular tissues are genetically programmed. For example, differences in the shape and size of the femur of a mouse, man, or an elephant are appropriately proportioned for the animal. Another example might be the lack of teeth in birds whose prehistoric ancestors possessed teeth.
2) Developmental outcomes are progressive and irreversible. There is a correct sequence of developmental changes that follow each other and these changes are not reversible. Even in crisis, for example during repair, there is no dedifferentiation of tissue [**17**]. Once differentiated, a cell type will produce particular progeny or specialized molecules, but the descendants are committed to the parental lineage. Therefore, to affect repair, undifferentiated stem cells must be activated (and/or even brought to the site: see **Fig. 1-4**), to provide the cells necessary for reparative processes.
3) Local environmental factors are of paramount importance in the rate and extent of development, maturation, and reparative processes. Such factors, which include cellular components and molecular products of those cells, may influence and hence determine the process of further cellular differentiation and expression. For example, a mesenchymal stem cell may differentiate into either an osteoblast or a chondrocyte by virtue of factors present in the immediate environment.

These three principles determine that, despite apparent similarities, the process of embryonic development is unique. Despite superficial similarities between the processes of bone formation, maturation, and repair, the mechanism of embryonic development cannot be recapitulated. The maturation and repair will take place in an environment profoundly modified by the existing structure. Therefore, an understanding of the embryological development of bone may explain how the tissue arises, but it cannot predict how the maturation process will continue, nor how regenerative mechanisms will operate.

The rest of this chapter will be devoted to a description of the process of embryonic development of bone: growth and maturation, the modeling and remodeling process, ectopic bone formation, and a brief discussion of the reparative processes.

3.1 Theories of bone formation

Until recently there has been a firmly established view that bone development occurred by one of two processes: either by direct transformation of connective tissue, known as intramembranous ossification, or by replacement of a previously formed cartilaginous model, endochondral or intracartilaginous ossification. In some bones it was accepted that both processes occurred simultaneously. In both intramembranous and endochondral ossification, the biochemical and the physiological processes were identical and involved activation of osteoblasts. The arguments for two separate methods of bone formation relied on the following observations:

- Endochondral ossification occurred where a rod of cartilage was seen to develop in the expected final position of the bone. This rod appeared to mimic the general shape of the adult bone and was considered as a precursor or template for the adult structure.
- There was an anatomical difference between bones that formed by endochondral ossification (predo-

minantly long bones and occasionally short and irregular bones) and those that developed by intramembranous ossification (flat bones of the skull and the subperiosteal layer of the diaphysis of long bones).
- Cranial/facial bones and bones in the rest of the body have distinct embryological origins: bones of the skull are derived from ectomesenchyme (neural crest cells) while other bones are derived from lateral plate mesenchyme [18].

Recently, however, considerable data has accumulated, primarily from work on the chicken tibia, to suggest that our concepts of these alternative approaches to ossification should be challenged [16].

3.2 Classical view of ossification

3.2.1 Intramembranous ossification

Bone develops within stromal connective tissue that is characterized by mesenchymal stem cells, connected by thin cell processes, lying in a matrix of haphazardly arranged collagenous fibrils. Immediately before ossification commences two changes are observed; the mesenchymal stem cells proliferate and start to differentiate, finally forming osteoblasts, and the intercellular matrix becomes more dense and homogeneous. These changes alone are sufficient to induce a suitable environment for early calcification to commence, and the mineral content of the matrix increases rapidly. The osteoblasts augment the process by producing more matrix that is calcified, and some of these cells will become trapped in the tissue and will transform into osteocytes. Until the bone has reached the final size, a layer of osteoblasts remain on the periosteal surface. The same process occurs for flat bones and on the periosteal surface of the diaphyses of long bones.

3.2.2 Endochondral ossification

Endochondral ossification occurs where bones elongate at a growth plate. This plate is arbitrarily divided into specific regions for descriptive purposes. At the epiphyseal front there is a layer of hyaline cartilage formed by cartilage cells, some of which may be embedded in matrix. The older cartilage cells begin to multiply and form into columns separated by wide parallel bands of interstitial substance. The cells are separated from each other by a thin capsule of matrix. These cells hypertrophy and incorporate stores of glycogen. Providing there are adequate concentrations of minerals available, the intercellular matrix then starts to calcify, particularly between adjacent columns of cells. This zone forms a provisional structural framework between the growth plate and the cancellous bone of the metaphysis. Loops of blood vessels then invade the connective tissue and penetrate into the vertical columns. The interstitial tissue is removed, leaving calcified vertical columns of matrix known as the primary spongiosa. This primary spongiosa is considered to be the necessary scaffolding upon which the bone matrix can be deposited. In this way the newly formed endochondral bone mirrors the cartilage model which it has replaced. The key feature of this hypothesis is that the cartilage model forms first and the bone is laid down onto that model. As bone matrix is laid down upon the primary spongiosa they are transformed into secondary spongiosa, a more permanent set of trabeculae. These will be modified by the joint action of osteoblasts and osteoclasts to form the thickened adult trabeculae, which are clearly visible upon gross examination of the cut surface of bone.

The pattern of mineralization at the growth plate can be clearly demonstrated by autoradiography and is of some interest. Comar et al. [19] showed that soon after calcium 45 (^{45}Ca) administration heavy deposits of radioactive ion are seen in the growth plate and adjacent trabecular bone of the metaphysis. Thirty days after ^{45}Ca administration, the radioactive content of the plate is relatively low and concentration in the trabecular bone is less than on day one. By 60 days, osteoclastic activity has removed and remodeled almost all the newly formed bone and the level of radioactivity observed is low in all areas.

Once an animal achieves skeletal maturity, bone stops growing in length and there is no further new formation of bone. The skeleton continues to be modeled and remodeled but the rate of change is considerably less than during the growth phase. Radioactive calcium introduced into bone at this stage may take years to be resorbed and removed. This underlines concerns about the hazards from certain radionuclides, for example strontium 90 (^{90}Sr) or strontium 89 (^{89}Sr), which have been shown to accumulate selectively in the skeleton [**20**, **21**].

3.2.3 Ossification revisited

Over the last decade, data have been emerging to support a reconsideration of the process of bone formation. Based on work on the chicken tibia, it is now proposed that the initial steps in the formation of long bones are different from those of previous theories. The critical differences between the two explanations of development are related to the role of the cartilage model that was thought to be a predeterminant of bone formation: the new hypothesis argues that a collar of bone-producing cells in the mid-diaphyseal region arises first. This collar gradually spreads to lie around the whole of the newly forming bone and defines the size of the cartilage rod (once thought to be the scaffold upon which the bone was laid down). Finally, the cartilage rod is then eroded and modified to form the medullary cavity of the adult bone.

The timing of events is summarized in **Tab. 1-1**. The critical mass of cells that will initiate the process of development is not the cartilage model but a group of four to six cells that are arranged as a stack in the mid-diaphyseal region. The stacked cells are arranged as a collar that will come to lie around a cartilaginous center, which will develop later. The cells of this collar are referred to as the stacked cell layer. These early stages include another important feature, the exclusion of vascular elements from the developing layers of cells. Vasculature is sandwiched between the collar of stacked cells and the chondrocytes that will form the cartilage rod that lies in a position similar to the final position of the adult bone [**22**, **23**]. The cells of the stacked layer will differentiate at the interface with the developing vasculature into osteogenic progenitor cells that will further differentiate into osteoblasts. These osteoblasts secrete the unique matrix, type-1 collagen-rich osteoid, that produces a rigid collar around the developing cartilaginous center. Caplan [**16**] speculates that this rigid collar forms a physical barrier for nutrients and other vascular-derived molecules that are diffusing into the avascular cartilage core. He speculates further that these physical limitations may initiate the observed hypertrophy of core chondrocytes. As the collar of osteoid begins to spread toward the ends of the long bone, the mid-diaphyseal region undergoes further mineralization and becomes bone.

The next stage of development may be the most significant. The stacked cell layer is invaded and penetrated by vascular elements that are positioned just outside the central region of the newly formed bone [**23**]. The capillaries invade through the osteogenic precursor layer and come to make a network of vessels over the first layer of mineralized bone. Lying between these invading capillaries and perpendicular to the first layer of newly formed bone, further osteoid struts are formed and are subsequently mineralized. Deposition of a second layer of bone, parallel to the first, completely surrounds the developing capillaries which are locked between the two layers of bone that are in turn connected by strengthening struts, the bony trabeculae. Fundamental to this process is the relationship between the capillary endothelium and the osteoblasts. Histological evidence suggests that these early osteoblasts have specific orientation with the base of the cells in contact with the capillary endothelium and secretion of osteoid occurring at the apex. The highly active secretory process, carried out by the osteoblasts, is clearly related to the direction of transport across the cell from the blood. Caplan [**16**] suggests that this unique relationship may explain the production of unique, large-diameter collagen fibrils which are observed in osteoid. The relationship between endothelium, its basement membrane and osteoblast may be of fundamental importance in our understanding of the process of development and might be significant for our appreciation of the role of vascular supply in regenerative/restorative processes in the adult. It has already been shown that the presence of vasculature at the site of breakage determines the method of repair. If there is a stable fracture site, and vasculature continuity can be established

between the broken fragments, then the mesenchymal repair blastema will differentiate directly into trabecular bone. If the fracture is not stable, an avascular repair blastema arises, characterized by the formation of a wedge of cartilage that plugs the gap between the fragments. Until recently, the key role of the vasculature at repair sites was thought to be related to nutrient supply, especially oxygen, to the area. The dependence of bone development on the endothelial/osteoblast relationship may indicate that the vascular elements in the reparative processes have an additional, and perhaps more significant, role to that of simply bringing extra nutrients and oxygen to the site of repair. Moreover, devising methods that stimulate this unique partnership between the lining cells of the capillaries and the bone-producing cells may be important developmental approaches for bone healing in the future.

The role of the cartilage model, which lies at the core of the developing bone and scaffolding theory of bone building, is now open for negotiation. While the collars of bone develop around the central group of cartilage precursor cells, these chondrocytes start to undergo differentiation and expansion. These changes in birds and mammals follow, not lead, the formation of the stacked cell layer of osteogenic precursor cells. Whether or not the chondrocytes begin to hypertrophy in response to starvation when the first layer of osteoid is laid down remains to be proven. Initially, these hypertrophied cells begin to secrete unique products such as large chondroitin

Table 1-1: The sequence of bone formation. A comparison between events in chicken, mouse, and where possible human fetus. (Data taken from Caplan AI, Pechak DG, Cell and Molecular Biology of Vertebrate Hard Tissues; 1988.)

	Sequential stage of development	Days of development		
		Chick	Mouse	Human
Stage 1	Formation of limb buds	3		
Stage 2	Commitment of mesenchymal cells to osteogenic lineage	4	12	40
Stage 3	Commitment of mesenchymal cells to chondrogenic lineage	4	13	40
Stage 4	Expression of phenotypic characteristics	4.5	14	40
Stage 5	Formation of cartilage core	4.5–7	14	40
Stage 6	Osteoprogenitor cells of the Stacked Cell layer	4.5	15	40
Stage 7	Production of mid-diaphyseal osteoid	6	15	50
Stage 8	Phase boundary between osteoid and cartilage core	6.5	15	50
Stage 9	Initiation of hypertrophy in cartilage core	6.5	15	50
Stage 10	Progressive proximal and distal spreading of osteoid layer	7.0–16	15–16	50
Stage 11	Mineralization of osteoid	7.5	15	50
Stage 12	Vascular invasion onto the mineralized collar	8	15	50
Stage 13	Cartilage hypertrophy culmination (cessation of synthesis of anti-angiogenesis factors)	9	14–15	50–55
Stage 14	Formation of vertical struts between capillaries	8.5	16	56–57
Stage 15	Initiation of second layer of trabecular osteoid	9	16	57–58
Stage 16	Marrow elements associated with vascular collar	8.5	16	60
Stage 17	Mid-diaphyseal invasion of first bone by osteoclasts	9	–	56
Stage 18	Vascular penetration and erosion of cartilage	9	15	56
Stage 19	Cartilage replaced by vasculature and marrow	9–14	16–17	60
Stage 20	Continued sequential formation of 12 more layers of trabecular bone	9–19	–	–
Stage 21	Dissolution of the first layer of bone by marrow elements	11	–	–

sulfate proteoglycan [24] and type-X collagen [25], but eventually they die; if they are rescued and maintained in an organ bath, they will continue to secrete these unique products for many months [26]. Furthermore, Caplan [16] argues that the histological appearance of developing cartilage is suggestive of pressure restrictions on growth within the cartilage core: chondrocytes in the center of the cartilage core are normally round cells, while those near the periphery are flattened at the bone–cartilage interface as if they have been compressed against the rigid walls of the collar of developing bone.

In mammals the core of hypertrophic cartilage is calcified for most bones, although in some sites the calcified cartilage is encapsulated with newly formed bone. The process is different in the chicken; the hypertrophic cartilage is not calcified or covered with bone. The next process is, however, common to mammals and birds. The cartilage core is replaced by marrow and vascular elements, not by bone [22]. This cartilage core, once considered to be the scaffold for new bone, is, however, a scaffold for the marrow cavity. It is therefore not surprising that the cartilage model at the core of the developing bone defines precisely the initial size of the marrow cavity of the bone.

The consequences of the shift in our understanding of the process of bone formation can be summarized:

- Formation of long bones and flat bones (endochondral and intramembranous ossification) occurs by the same process.
- The relationship between endothelial cells of invading vasculature and the first osteoblasts is fundamental to the process of development and may be vital for reparative processes.

4 Ectopic bone formation

Cells located in sites removed from bone surfaces, in extraskeletal sites, have the capacity for true bone formation [27–29]. The differentiation of an unspecialized mesenchymal cell population into bone tissue is initiated by a process known as bone induction. Huggins [30] demonstrated bone induction in a series of classic experiments almost 60 years ago by transplanting urinary epithelium into various connective-tissue sites in dogs and rabbits. Subsequently, other living epithelial cells were found to have similar properties [31, 32]. Transplanting bone fragments into non-skeletal sites also results in the induction of bone formation, indicating that bone tissue contains endogenous factors that regulate and control the formation of ectopic bone. Goldhaber [33] demonstrated that normal mouse bone synthesizes and secretes a bone-inducing factor capable of inducing bone formation. A similar substance was later discovered in certain mouse and human osteosarcomas [34–37]. Urist [38] was the first to show that devitalized bone contains an osteoinductive agent, which he named bone morphogenic protein (BMP).

It is important to identify and characterize osteoinductive agents as these would allow basic studies on osteogenic induction and osteogenesis at the cellular level and, more importantly, allow an assessment of their mechanisms of action in abnormal bone growth and healing processes. Based on the original techniques for producing soluble fractions containing osteoinductive factors [39], there have been several attempts at biochemical isolation of the materials [39–42]. These factors from a variety of sources have been shown to be non-collagenous proteins of low molecular weight; for example, human BMP and bovine BMP are said to have molecular weights of approximately 18,000 and to have characteristics of acidic proteins [43–45]. These substances have not been sequenced or, as Triffitt [2] suggests, if they have been sequenced, the results are closely guarded commercial secrets. Despite similarities in size between various osteogenic factors, there may be differences in composition; for example, osteosarcoma-derived BMP is a basic protein [2]. Levels of monoclonal antibodies to the major protein in purified bovine fractions with BMP activity in normal patients have been compared with those in patients affected by a variety of bone diseases [44, 46]. Despite clear differences in serum between individuals, data on the full characterization of the antibodies are not available.

Histologically, formation of bone from a transplanted bone chip resembles the classic picture of endochondral ossification. The initial phase is characterized by attraction of mesenchymal stem cells to the site of implantation. These stem cells surround

the chip and within 1–3 days there is a powerful wave of mitogenic activity followed by differentiation into cartilage around the bone fragment. The cartilage becomes calcified, and new bone forms. It has been accepted that this process demonstrates the cartilage model system for bone formation, but closer inspection of the temporal events has revealed otherwise. Caplan [**16**] reports that there is a layer of osteogenic cells that form a sheet covering the bone chip and that this layer of cells, in intimate contact with invading capillaries, forms the first osteoid which is mineralized onto the surface of the bone fragment. The hypertrophic cartilage is, however, replaced by marrow, and there are accounts of marrow formation associated with these bone chips [**47**].

5 Development and maturation of bone

There are differences in the timing of appearance of secondary centers of ossification, their positions and rates of growth between species, but comparative analysis can be useful in establishing trends. In man, with a gestation period of 275 days, the ossification centers can be detected initially at 63 days, toward the middle of the first trimester of pregnancy. The centers develop rapidly and their position and extent for the eleventh week of gestation is shown diagrammatically in **Fig. 1-5a**. Although centers of ossification develop in a similar pattern in the dog, they are found much later. Gestation in the bitch lasts 63 days but the centers of ossification do not appear until at least day 28 of pregnancy. These centers are shown for day 33 of pregnancy in **Fig. 1-5b**. In consequence, during the second half of pregnancy, fetal puppies undergo massive skeletal development that continues into the neonatal period. The rate of development is clearly related to the immediate functional needs of the neonate. Calves, foals, fawns, and many other animals are born with all of their secondary ossification centers actively engaged in growth and almost all of the appendicular and axial skeleton at least partly ossified. These newborn animals are expected to stand within minutes or hours of birth, follow their mothers, and even run to escape predators. Marsupials show perhaps the most spectacular form of differential development of the skeleton. The minuscule fetal marsupial is born with fully functional weight-bearing forelimbs and axial skeleton as far distal as the first few thoracic vertebrae. The remaining caudal vertebrae and the primitive limb buds that represent the final position of the hindlimbs are hardly developed at all. In this partly developed condition, they crawl, with the aid of their head, neck, and forelimbs, from the vulva into their mother's pouch and attach to a waiting nipple where they can continue growth.

5.1 Axial skeleton

5.1.1 Skull

There are many modifications and adaptations of the skull throughout the animal kingdom, with some spectacular evolutionary switches in the function of various components of the skull. For example, Hamilton and Mossman [**48**] showed that the ear ossicles, which are used to transmit sound waves in higher mammals, are derived from structures that support the gills in primates and chordates, and form part of the jaw in fish, reptiles, and amphibia.

Within a species there can be considerable variation in the shape and size of the skull. For example, there are racial differences in facial bone structure in man [**49–53**], and there have even been contentious claims of racial traits and even abilities associated with cranial vault size, which have been discredited. In cattle, there are vast differences in breed, size, and shape of the head, perhaps best exhibited by comparing beef and dairy breeds, or polled and horned breeds. It is, though, in dogs, where man's intervention has exaggerated the differences by selective breeding, that such differences can be seen so clearly. Consider the difference between the wide, squat-nosed, massive, heavy face of the Bulldog, a brachycephalic breed, and the elongated, fine, pointed head of a dolicocephalic breed such as the Afghan. It is interesting to note that these clear-cut differences, between the skulls of brachy-

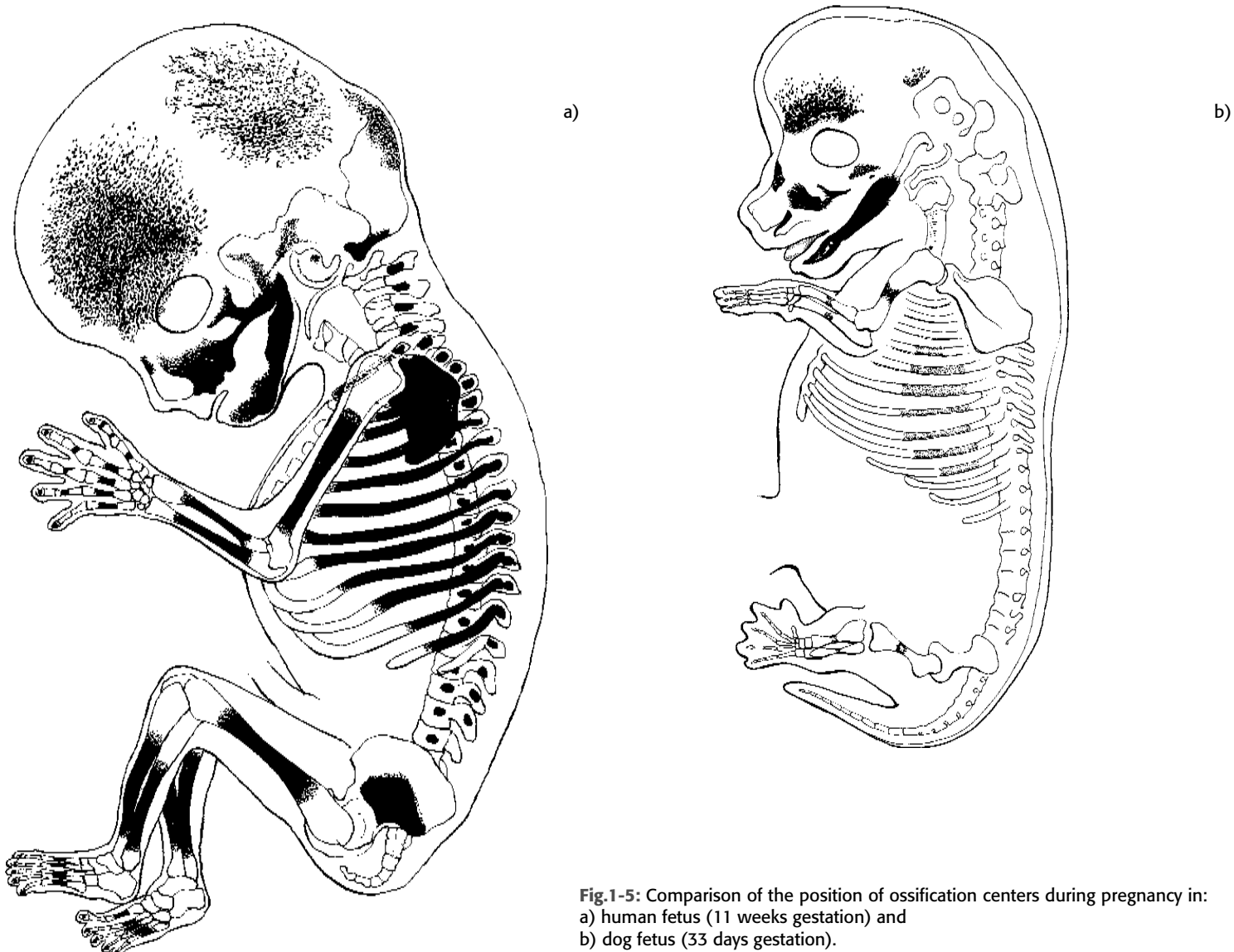

Fig.1-5: Comparison of the position of ossification centers during pregnancy in:
a) human fetus (11 weeks gestation) and
b) dog fetus (33 days gestation).
Note the relatively advanced state of ossification observed in the human fetus.

cephalic and dolicocephalic breeds, are not present at birth. Puppies are generally born with a common, basic head shape that will undergo genetically determined modifications as the puppy matures.

In general, the larger the head at birth, the less the bones of the skull are completely ossified. This is observed to the greatest degree by comparing the skull of a newborn child with that of a newborn puppy. The head of a human baby is approximately a quarter of the total length of the newborn. Delivery of the head represents the greatest hurdle during birth in humans and it is vital that the head can be molded to the shape of the birth canal. In consequence, the cranial vault is not completely ossified in newborn infants and patent fontanelles are present. Fontanelles are also seen during development in other species but the gaps between cranial bones have been closed and many, if not all, of the bones of the skull have undergone ossification by the time of birth. After delivery, a child's head progressively decreases proportionally in size compared with the rest of the body until it represents only a sixth or perhaps a seventh of the total body length of the human adult. There is less difference in the comparative size of the neonatal dog and the adult. However, some species, particularly the dolicocephalic breeds, will show substantial elongation of the facial bones during early postfetal life.

5.1.2 Vertebral column

Development of the vertebral column in higher vertebrates is initiated by the axial notochord. This primitive structure is surrounded by mesoderm during early embryonic life and condenses into sections to form somites. From these somites concentrations of mesenchyme develop, known as sclerotomes, that will form the vertebrae and, where appropriate, the ribs. The basic shape of individual vertebrae is similar, irrespective of whether they will develop into cervical or lumbar vertebrae. Typically, each vertebra has three centers of ossification, one for the centrum and one for each of the two neural arches. From the midline fusion of these two arches the dorsal spinous process will develop. Later, the transverse and costal (if appropriate) processes will develop from the position where the ossification centers of the neural arches fuse to the developing centrum. For each region of the vertebral column, with the exception of the cervical region (where there are always seven vertebrae with only one or two exceptions, even for the long-necked giraffe), higher mammals have different numbers of vertebrae, but the characteristic shape of a vertebra from each region is consistent across species. For example, lumbar vertebrae have well-developed transverse processes to support the lateral and ventrolateral abdominal wall; the thoracic vertebrae have more pronounced dorsal spines and costal foveae for articulation with the ribs. The first two cervical vertebrae are, however, different from the others in their regional grouping: the body of the atlas (cervical vertebra one, C1) fuses to the body of the axis (cervical vertebra two, C2) forming the dens.

The appearance of the three ossification centers for each vertebra does not occur simultaneously, nor is a craniocaudal wave of development observed [**54**]. In general, the centers develop first and there is logical sequence from C1 through to thoracic vertebra seven (T7). Initiation of the centers for these vertebrae is rapidly followed by the appearance of the centers for the neural arches of the same segments. Then, for an unknown reason, the sequence is interrupted, and ossification centers for the caudal (coccygeal) vertebrae five to seven (Co 5–7) appear, followed by their respective arches. The craniocaudal sequence of development then resumes in the midthoracic region.

Lateral costal processes develop from the precursor thoracic vertebrae into the spaces between developing myotomes. These will separate from the developing vertebrae and form the ribs, each with a separate true articulation with the vertebrae at the proximal end and a cartilaginous articulation with the sternum at the distal end.

In addition to its functional support for the animal, flexibility of the spine is a prerequisite for locomotion. There are approximately 40 joints throughout the vertebral column whose movement is limited by conformation of the articular surfaces and ligaments involved. Most of these joints are limited to flexion, extensions and lateral movement. Only the occipito-atlantoaxial unit is different. Together, the articulations between these bones function more like a universal joint and afford greater ranges of movement. The unique

movements, exhibited by these cranial articulations of the spine, are related to the specialized form of the bones and articulations involved. There are differences between species but, in general, the same basic shapes can be seen in all species. The occipital bone terminates at the occipitoatloid joint by two condyles with very large surface areas permitting considerable excursions of movement. The atlas, unique among vertebrae by its lack of a body, has two large lateral processes, wings, that serve for muscle attachment. In turn, the atlas articulates with condyles of the axis and rotates around the dens of the axis. The pivoting movement between C1 and C2 determines whether the face can be rotated 180° (man), greater than 240° (owls), or less than 100° (cattle).

5.1.3 Ribs

Embryological origins of the ribs have been discussed above. There is considerable variation between species in the number of ribs present and the presence or absence of false ribs (not connected to the sternum). In general, higher mammals possess nine ribs that are connected directly to the sternum and between three to eight ribs that are either linked to the sternum by cartilage or may be completely unconnected. Together, the double rows of ribs form the bony cage that protects the thoracic viscera. The shape of this thoracic cage differs according to the posture and size of the animal and reflects the stresses exerted on the thorax.

5.1.4 Sternum

The sternum develops from two midline ventral (anterior in man) condensations of mesenchyme in the thoracic region of the embryo. Each side of the sternum is known as a hemisternum and is curved in two directions: boat-shaped along the ventral surface of the embryo, and curved away from the midline as the condensation progresses caudally. As the ventral surface of the embryo closes, so the two hemisterna move closer together and fuse, at least at the cranial end. There is considerable species difference in the degree of fusion observed. Laterally, the hemisterna attract the distal ends of the developing ribs, but they do not fuse in a craniocaudal sequence. Usually, ribs 2–7 fuse before the first rib unites with the sternum, followed by the last two true ribs. Anomalies of closure of the two hemisterna, and of fusion of the last two true ribs, are relatively commonly occurrences.

As the sternum grows there is considerable variation in shape and size between species. In man, the sternum expands to form a flat plate of bone that might be considered important in protection of the thorax. The same structure is elongated, thin, and clearly reflects its segmental origin in the dog, while in the horse and cow the sternum retains its original boat-shaped appearance and even grows to form a ventral projection akin to a keel that serves for muscle attachment.

5.2 Appendicular skeleton

For orthopedic purposes, postnatal development of the appendicular skeleton is of paramount importance. Centers of ossification in man are relatively consistent over the time of their appearance and fusion, which means that it is possible to make predictions about bone length and assessment of age with reasonable accuracy. The same is not true for dogs. Breed variation in size and shape makes it impossible to use bone length as an accurate guide to age. Sumner-Smith [**55**] compares the time of fusion of epiphyses throughout the skeletal system with age. This produces a reasonable correlation, but there is still considerable variation in the earliest and latest time fusion for one particular epiphysis (**Tab. 1-2**). There appears to be a relatively consistent chronological order to the sequence of fusion. It may be useful to list a number of factors that cannot be related consistently to the timing of fusion of epiphyses in dogs; for example, variation between siblings is commonplace, there is no predominance shown by male, female, or neutered animals, and breed size does not effect time of fusion.

Table 1-2a: A comparison of the time of appearance of ossification centres and growth plate fusion in man and dog—pectoral limb.

	Man				Dog	
	Ossification centre		Growth plate fusion		Ossification centre	Growth plate fusion
Scapula						
Coracoid	1		18–21		–	–
Acromion	15–18		18–19		–	–
Glenoid cavity	18		19		–	–
Supraglenoid tubercle	prenatal		15		12 wk	5 mo
Clavicle	17		18–24		absent	–
Humerus						
Proximal						
Head	fetal		centres fuse together 4–6		only one centre	
Greater tubercle	6 mo–2 3 mo–1		fuse to shaft 19–21		present at birth	fuse to shaft 13–14 mo
Lesser tubercle	3–5		18–20			
Distal						
Medial epicondyle	7	5	18	15	3–4 mo	fuse to shaft
Trochlea	9	8	fuse together at puberty		one centre	5–8 mo
Lateral epicondyle	12	11	fuse to shaft		prenatal	
Capitulum	5 mo	4 mo	17	14	prenatal	
Ulna						
Olecranon	10	8	15–17	14–15	3–4 mo	5–9 mo
Distal epiphysis	6	5	19	17	3–4 mo	6–8 mo
Radius						
Head	5	4	13–17	14–15	prenatal	5–8 mo
Radial tuberosity	10–12		14–18		absent	partial fusion to ulna 11 mo
Distal epiphysis	1		19	17	prenatal	6–9 mo
Carpus						
Accessory	6 mo		4		3–4 mo	5–6 mo
Radial	6		–		3 mo	–
Intermediate	4		–		3 mo	–
Ulna	12		–		3–4 mo	–
I	5		5		3 mo	–
II	4		–		3 mo	–
III	6 mo		–		3 mo	–
IV	6 mo		–		3 mo	–
Metacarpals						
I	2	$1^{2}/_{3}$	14–21		absent	–
II–V	$1–1^{1}/_{2}$		14–21		3–4 mo	5–8 mo
Phalanges						
Proximal	5 mo–$2^{1}/_{2}$		14–21		3–4 mo	5 mo
Middle	5 mo–$2^{1}/_{2}$		14–21		3–4 mo	5 mo
Distal	5–2 (except I $1^{1}/_{2}$ 1)		14–21		3–5 mo	5 mo

Table 1-2b: A comparison of the time of appearance of ossification centres and growth plate fusion in man and dog—hip bones.

	Man		Dog	
	Ossification centre	Growth plate fusion	Ossification centre	Growth plate fusion
Hip				
Acetabular	10–13		6–8 wk	4–6 mo
Ischium	60 wk (fetal)		25 wk (fetal)	
Pubis	60 wk (fetal)	fuse at puberty (12–13)	20 wk (fetal)	fuse 1–2
Illium	60 wk (fetal)		10 wk (fetal)	
Iliac crest	puberty		4 mo	1–2$^{1}/_{2}$
Ischial arch	13–15		5–8 mo	8–12 mo
Ischial tuberosity	13–15		2$^{1}/_{2}$–4 mo	6–10$^{1}/_{2}$ mo
Symphyseal cartilage	13–20		4–10 mo	fusion symphysis (1–5)

Table 1-2c: A comparison of the time of appearance of ossification centres and growth plate fusion in man and dog—pelvic limb (excluding hip bones).

	Man				Dog	
	Ossification centre		Growth plate fusion		Ossification centre	Growth plate fusion
Femur						
Greater trochanter	3		16–17		3–4 mo	9–11 mo
Lesser trochanter	12	11	16–17		3–4 mo	9–10 mo
Head	4 mo		17–18	16–17	3–4 mo	6–9 mo
Distal epiphysis	36 wk (fetal)		18–19	17	3–4 mo	6–8 mo
Tibia						
Proximal epiphysis	40 wk (fetal)		18–19	16–17	3–4 mo	6–11 mo
Tibial tuberosity	7–15		19		3–4 mo	8–11 mo
Distal epiphysis	6 mo		17–18		3–4 mo	5–11 mo
Fibula						
Proximal epiphysis	4	3	18–20	16–18	3–4 mo	6–10 mo
Distal epiphysis	1	9 mo	17–18		3–4 mo	5–8 mo
Tarsus						
Calcaneus	24–26 wk (fetal)	12–22			P.N.	4–7 mo
Talus	26–28 wk (fetal)					
Navicular	2					
Cuboid	40 wk (fetal)	variable			variable	
Cuneiforms I–II	1–2					
III	3–6 mo					

All times are given in years except where indicated.

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5.2.1 Pectoral limb

Scapula

The position of the scapula and its relation to the thorax differs substantially in man from other animals. It forms part of the true pelvic girdle while in quadrupeds the pectoral limb is attached to the axial skeleton by a synsarcosis. In both man and dog, the body of the scapula is present at birth, derived from one major center of ossification. This major center also gives rise to the spine and acromion of the scapula. Shortly after birth, a second center appears in man [56] and dog [57], which gives rise to the supraglenoid tubercle. In dogs fusion takes place slowly with the rest of the scapula, and the cartilage plate is usually eroded by 28 weeks postpartum. In cats and horses, there is another secondary center of ossification adjacent to the glenoid cavity which fuses shortly after birth.

Clavicle

This bone is an important part of the pectoral girdle. It is therefore present in many quadrupeds that climb or dig. Most of the common domestic species only possess a bony (cat) or cartilaginous (dog) remnant of this bone, which is intercalated into the brachiocephalic muscle. In man, an ossification center for the clavicle is among the first to develop in the fetus [58]. The secondary ossification center, however, develops much later (at about 11–14 years of age) on the sternal end of the bone.

Humerus

The shaft of the humerus is present at birth. Arey [56] reports that the shaft is present as early as the seventh week of pregnancy in man. In addition, an ossification center is present at the head of the humerus at birth. Appearance of this center during fetal life can be used to identify accurately fetal age since it develops in the human fetus during week 38 of gestation [59]. There is species variation in the number of centers of ossification present. In man, the proximal center divides during childhood to give rise to two centers that will form the greater and lesser tubercles of the proximal end of the bone. These centers fuse together before uniting with the shaft of the humerus. The major increase in bone length, seen during childhood, occurs at the proximal end of the bone [58]. Three centers of ossification develop during childhood for the distal end of the shaft. These correspond to the medial and lateral parts of the distal condyle and one for the medial epicondylar region. In dogs, there is only one proximal center of ossification for the humerus. From this single area the greater and lesser trochanters are formed. The cartilaginous growth plate, between the proximal center and the shaft, remains intact until the 43rd week of life. This might suggest that in dogs the major region for growth in length of the humerus occurs at the proximal end of the bone, similar to that reported in man. By 51 weeks only remnants of the plate are seen and gradually, over the next 8 weeks, the plate is removed completely. Distally, three centers develop which start to fuse from the 21st week of life onwards and fusion is completed by the 33rd week.

Radius

Initially, this bone appears as a long cylinder, which develops spherical-shaped centers of ossification at both ends in early childhood (man), or within the first 4 weeks (dog). Gradually, the centers broaden out and assume the characteristic shape of the adult bone. Fusion occurs in children aged 8–11 years and in puppies between 45–47 weeks.

Ulna

Formation of the ulna is more complex. In many of the domestic species the bone is partly or completely fused to the radius during development. The most extreme example is the horse where the olecranon and proximal third of the bone are present: the latter decreases substantially in size distal to the elbow joint and is completely fused to the radius from birth. In man and dog, the ulna is present at birth as a long cylinder on the caudolateral aspect of the rudimentary radius. Shortly after birth, the characteristic semilunar trochlear notch develops at the proximal end and starts to interact with the developing proximal radius and distal humerus to form the elbow joint.

In dogs, proximal and distal centers of ossification appear at 8 weeks. The distal epiphysis grows rapidly in an uneven manner; two spurs of developing bone grow on the medial and lateral sides of the ulnar metaphysis. This uneven rate of growth continues and by 12 weeks the cartilage separating the metaphysis and epiphysis has adopted a V-shaped appearance. The distal epiphysis swells and becomes larger in diameter than the shaft of the ulnar diaphysis. Complete fusion of the distal center is achieved by the 47th week while the proximal epiphysis fuses to the shaft earlier, during the 37th week. In both man and dog small foci of ossification associated with the anconeal process have been identified. Almost as soon as these foci appear fusion starts to occur, although there are many documented cases where failure of fusion leads to a pathological, non-united anconeal process which will be associated with elbow dysplasia.

Coordination between the growth rates of the radius and ulna are important in the normal development of the forearm. In animals where the bones are linked together there are fewer reported conditions of uncoordinated growth, but in man and dog premature closure of one of the growth plates will result in malformation of the forearm, and possibly the elbow and carpus. Problems associated with premature or failed closure of the proximal ulna plate are relatively common but will not affect conformation of the forearm. Premature closure of the distal growth plate of the ulna is the most common condition that will distort the bones; the manus will deviate laterally and the forearm will curve. Premature closure of the distal plate of the radius is less common and is not usually associated with bowing of the forearm: quite the contrary, the forearm is reported straighter than normal, but the patient experiences elbow joint pain. (The elbow pain is usually greater in quadrupeds as the condition is exacerbated by weight bearing.) Failure of closure of the proximal radius growth plate is rare: accompanied by no change in the conformation of the forearm, an increase in the humeroradial space that can be detected radiographically and joint pain, especially upon palpation.

Carpus

Carpal centers of ossification are not present in either man or dog at birth. There is a similar sequence for the appearance of these centers in both species but a considerable difference in time scale. Centers for the intermediate and accessory carpal are the first to develop, followed quickly by centers for the other five bones. There is partial fusion of the bones in the carpus of the dog, and the initially separate centers of ossification for the radial and intermediate carpal bones in the dog quickly fuse and are completely united by the 12th week of life. A second center of ossification appears for the accessory carpal bone in both man and dog. In general, complete fusion of the epiphyses has occurred by the age of 6 months in dogs and by 10 years in children.

Manus

Shafts representing the rudimentary metacarpals and phalanges are present at birth for the major digits present (four in dog, five in man). Each of the bones and the sesamoids that develop subsequently on the palmar aspects of the metacarpophalangeal joints develop one center of ossification. In general, the metacarpal centers remain active for longer than the phalangeal centers, but there is great variability in the timing and sequence of closure of the plates between digits.

5.2.2 Pelvic limb

Hip bones

Despite major differences in the shape and form of the hip bones between species, there are underlying trends that outline development. Considering the hip bones of dog and man, the most notable differences are the lateral divergence of the wings of the ilia in man compared with the almost cranio-caudal direction in the dog. Nevertheless the bones start to form in much the same manner. At birth, in both species, there are three major centers of ossification that will develop into the three major bony components of the pelvis: ilium, ischium, and

pubis (paired structures). A center develops during weeks 6–7 in dogs, or during the third trimester in man, which will form the acetabular bone. The components of the acetabulum fuse together and other centers of ossification for parts of the ischial tuber, iliac crest, and eventually the symphysis develops. Complete ossification of the pelvic symphysis occurs up to age 6 in dogs and during late teens or early twenties in man.

Femur

Again, the shaft of this bone is present in almost all species at birth. For animals that are expected to stand and walk within minutes or hours of birth other components are also present, such as proximal and distal centers of ossification, which allow contact to be made and rudimentary joints to be established with respective bones. In dogs, an epiphysis develops within 2 weeks of birth at either end of the shaft. By 8 weeks a further pair of centers develops at the proximal end of the femur which will form the greater and lesser trochanters. The first proximal center forms the head of the femur and takes a considerable length of time to fuse to the shaft. This period and the integrity of the head are critical for normal conformation to be attained. The single distal center develops into the complex trochlea, condyles, and epicondylar regions of the distal end of the femur. Closure of the growth plates occurs between the 41st and 47th week in dog. At approximately 32 weeks of life the center for the patella, followed 20–30 weeks later by small foci for the fabellae, develops. There are subtle differences in the shape of the human femur, including a longer femoral neck, wider distal condylar region, and less pronounced lesser trochanteric region, but the pattern and sequence of development is similar. The timescale of development is extended into late childhood for complete fusion to occur.

Tibia

With the shaft ossified at birth, the tibia grows in length with the appearance of a single proximal and a distal epiphysis. There are peculiarities about the changes observed at both these centers. The proximal center develops first and a small notch appears in the cranial aspect of the center. Development of the distal epiphysis then occurs, rapidly followed by the appearance of a third center which is responsible for the tibial tuberosity. Ossification at the distal center does not occur by circumferential growth but seems to develop primarily on the medial aspect of the bone and spreads around the periphery of the cartilage plate. Fusion of the three plates usually occurs first in the distal center, followed by the tibial tuberosity and lastly by the proximal center, although they may be in various stages of closure simultaneously.

Fibula

Animals from species that retain a fibula during development are born with an ossified shaft. Following a now fairly familiar pattern, two centers of ossification appear at either end of the bone: the proximal epiphysis appears first and is the first to fuse, the distal appearing and fusing slightly later. The central portion of the diaphysis in the dog fuses for a short but individually variable distance to the developing tibia. In most breeds of dog the shaft of the fibula is straight but is twisted laterally around the tibia in man [**58**].

Tarsus

There are striking differences between the tarsal regions of bipedal and quadrupedal animals. The plantigrade locomotion of man produces concussive forces on the tarsal region that are not experienced by quadrupeds. However, the pattern of ossification and development of the two regions is similar between bipedal and quadrupedal animals; for example, animals are born with the centers developed for the calcaneus and talus, followed shortly after birth by centers for the central, third, and fourth tarsal bones. The appearance of a second growth area for the calcaneus usually occurs at the time that centers for the first and second tarsal bones develop. Many of the domestic species show varying degrees of fusion between tarsal bones. Like their counterparts in the carpus, the early development of separate centers for each of the tarsal bones is rapidly followed in these species by immediate fusion of these centers giving rise, for example, to a fused central and fourth tarsal in the ox, or a fused first and second tarsal in the horse.

Pes

With the exception of the longer length of the metatarsal bones in most species, in comparison with the metacarpals in the same animal, the sequence and rate of development of the pes is similar to that described for the manus.

6 Bone modeling and remodeling

Overall conformation of adult bone is determined genetically. Once maturity is achieved bone ceases to grow, but mechanical stresses of weight bearing, muscle attachment, and applied loads will all result in constant adaptation of the internal structure and external appearance of bone. There is a vast panoply of factors that are known to affect bone growth and modeling; some of these are more important in the initiation of bone growth, others in the growing process itself or in the modeling process, while some are vital for reparative processes (see **Chapters 7** and **9**).

Bone resorption, the primary function of osteoclasts, occurs predominantly on the endosteal surface. Tunnels are eroded into the bone at right angles to the shaft and are occupied by osteoblasts and vascular elements. Quickly, layers of lamellar bone are laid down and the osteoblasts are stranded and enclosed in matrix, becoming osteocytes within lacunae. In this way a progression of osteons are formed, each layer breaking through established bone.

The mechanics of resorption are not completely understood. Osteoclastic activity is fundamental to the process. These cells are present, at all times, on the surfaces of bone; yet bone is not continuously eroded and resorbed at all sites. This argues that: Either the osteoclasts are not always active and have to be goaded into action or the bony structures are protected by a lining of condensed connective tissue or perhaps a very thin layer of bone-lining cells. The last of these possibilities is considered most likely [**60**]. The following sequence is suggested:

- The barrier which protects the bone itself has to be removed.
- The exposed matrix attracts mononuclear phagocytes to the bone surface.
- Resorption is initiated.
- The mononuclear phagocytes fuse together and form histologically recognizable osteoclasts.

The exact process of resorption has yet to be elucidated. The osteoclasts have a ruffled or brush border in contact with the bone surface and it is suggested that the following process might occur:

- Components of the bone matrix are released first by a variety of hydrolytic enzymes, including collagenase.
- These mineral components and collagen fragments are phagocytized by the osteoclast.
- Complete enzymatic dissolution of the matrix is achieved within osteoclastic vacuoles.

7 Bibliography

Blue references indicate links to abstracts of articles available online:
http://www.aopublishing.org/BONE/1.htm

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